Bee T

Transformation of E.*coli*

1. Remove competent cells from freezer and allow to thaw on ice for 10 min
2. Take care not to disturb the competent E.*coli*: do not vortex them or pipette them up and down.
3. Add 50 µL of thawed competent cells and then 1 - 2 µL of the re-suspended DNA to the labeled tubes. Make sure to keep the competent cells on ice.
4. Incubate the cells on ice for 30 minutes.
5. Heating shock the cells by immersion in pre-heated water bath at 42°C for 60 seconds. A water bath improves heat transfer to the cells.
6. Incubate the cells on ice for 5 minutes.
7. Add 950µl of SOC broth (make sure that the broth does not contain antibiotics and is not contaminated)
8. Incubate the cells at 37°C for 2 hours while the tubes are rotating or shaking.
9. Prepare two dishes with LB agar and the appropriate antibiotic(s) with the part number, plasmid, and antibiotic resistance. Centrifuge under 4000g, 4min and remove 800 µl supernatant medium and plate 200 µl of the transformation onto the dishes, and spread. This helps ensure that you will be able to pick out a single colony.
10. Incubate the plate at 37°C for 12-14 hours
11. Always keep agar plates upside down so that drips of condensation and falling debris do not contaminate them.